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Previews

Targeting Mechanisms in Myelinated Axons: Not All Nodes Are Created Equal

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A recent *Neuron* paper by Zhang et al. (2012) reveals how ion channels and adhesion molecules essential for rapid nerve conduction in vertebrates are differentially targeted to nodes of Ranvier. Moreover, distinct mechanisms regulate initial clustering and maintenance of specific nodal components.

Elaborate architectures underlie the specialized functions of many cell types. Neurons provide a striking example, with opposite poles of the cell bearing structures that receive and transmit information (dendrites and axons, respectively). The axon is itself organized into specialized subdomains, including the axon initial segment, nodes of Ranvier, internodes, and others. The precise trafficking and localization of specific ion channels, adhesion molecules, and cytoskeletal components to the appropriate domains is essential for saltatory conduction by myelinated axons. A paper by Zhang et al. (2012) in a recent issue of *Neuron* reveals that extracellular interactions initially localize adhesion molecules to nascent nodes, and that a different mechanism involving intra-axonal transport acts later to localize ion channels and other components to the mature node.

In vertebrate peripheral nerves, most of the myelinated axon comprises internodes wrapped by myelinating Schwann cells. The voltage-gated sodium channels (VGSCs) that propagate the action potential reside at the short unmyelinated gaps between Schwann cells, called nodes of Ranvier. Each node is flanked by paranodes, sites of specialized junctions between axons and Schwann cells. A number of studies have defined the molecular composition of nodes of Ranvier and identified molecules that localize VGSCs to nodes. The immunoglobulin cell adhesion molecule Neurofascin-186 (NF186), for instance, is necessary for VGSC localization at nodes of Ranvier in the peripheral nervous system (PNS) (Sherman et al., 2005; Zonta et al., 2008; Thaxton et al., 2011). The cell biological mechanisms that target NF186 and other key proteins to nascent and mature nodes have, however, remained unclear. Before mature myelin sheathes are formed, Schwann cells extend processes along the length of axons, and, in some regions, form heminodes, transient structures defined by the appearance of nodal components accumulated next to the tip of a Schwann cell's process (Figure 1). One model suggests that the growth of neighboring Schwann cell processes toward one another actively clusters nodal proteins already diffusely localized in the associated axonal membrane (Pedraza et al., 2001).

Zhang et al. provide clear evidence that some axonal proteins are indeed localized to the node via extracellular interactions with Schwann cells, whereas others are not (Figure 1). Axons separated from their cell bodies, but manipulated to prevent degeneration, have impaired axonal transport but nonetheless can support myelination and nodal localization of specific cell adhesion molecules (CAMs), including NF186. These axons, however, fail to target ion channels or cytoskeletal components to nodes. Similarly, specific disruption of vesicular transport (following brefeldin A treatment) prevents ion channels and cytoskeletal components from reaching the node, but leaves CAM targeting intact. Compelling extracellular surface labeling experiments confirm that NF186 already in the axon membrane becomes localized to nascent nodes by extracellular interactions with Schwann cells.

In an additional series of experiments, Zhang et al. reveal that different mechanisms deliver NF186 to nascent nodes compared to mature nodes (Figure 1). They create a series of transgenic mice with neurons expressing fluorescent fusion proteins that include full-length wild-type NF186 and chimeras of NF186 and ICAM, a lymphocyte CAM that has a similar structure and molecular weight to that of NF186, but does not exhibit any specific axonal localization when heterologously expressed in neurons. Analyses of these chimeric proteins show that the NF186 ectodomain is necessary and sufficient for targeting to nascent nodes in vivo (Figure 1). Interestingly, after the paranodal axon-glial junction forms (defined by colocalization of axonal and glial CAMs; Figure 1), only proteins with an intact NF186 cytoplasmic C-terminal domain are correctly targeted to the mature node, where their localization is stabilized by interactions with ankyrin G. These data suggest that NF186 targeting to nodes occurs in two distinct phases. The first is mediated by its ectodomain and presumably occurs via interactions with the growing tips of myelinating glia, and the second is mediated by its C-terminal domain and occurs via vesicle transport, as defined in dynamic imaging of cells in culture (Figure 1).

Previous studies have implicated gliomedin, a protein that binds NF186 and localizes to the growing tips of myelinating Schwann cells, as a glial cue that promotes formation of the node (Eshed et al., 2005). A more recent analysis of knockout mice, however, showed that gliomedin is not required for NF186 or VGSC localization to PNS nodes of Ranvier, although clustering at heminodes was disrupted (Feinberg et al., 2010). The new demonstration by Zhang et al. that two distinct mechanisms localize NF186 to nodes of Ranvier provides a model of gliomedin function during nascent node assembly. The interaction between the NF186

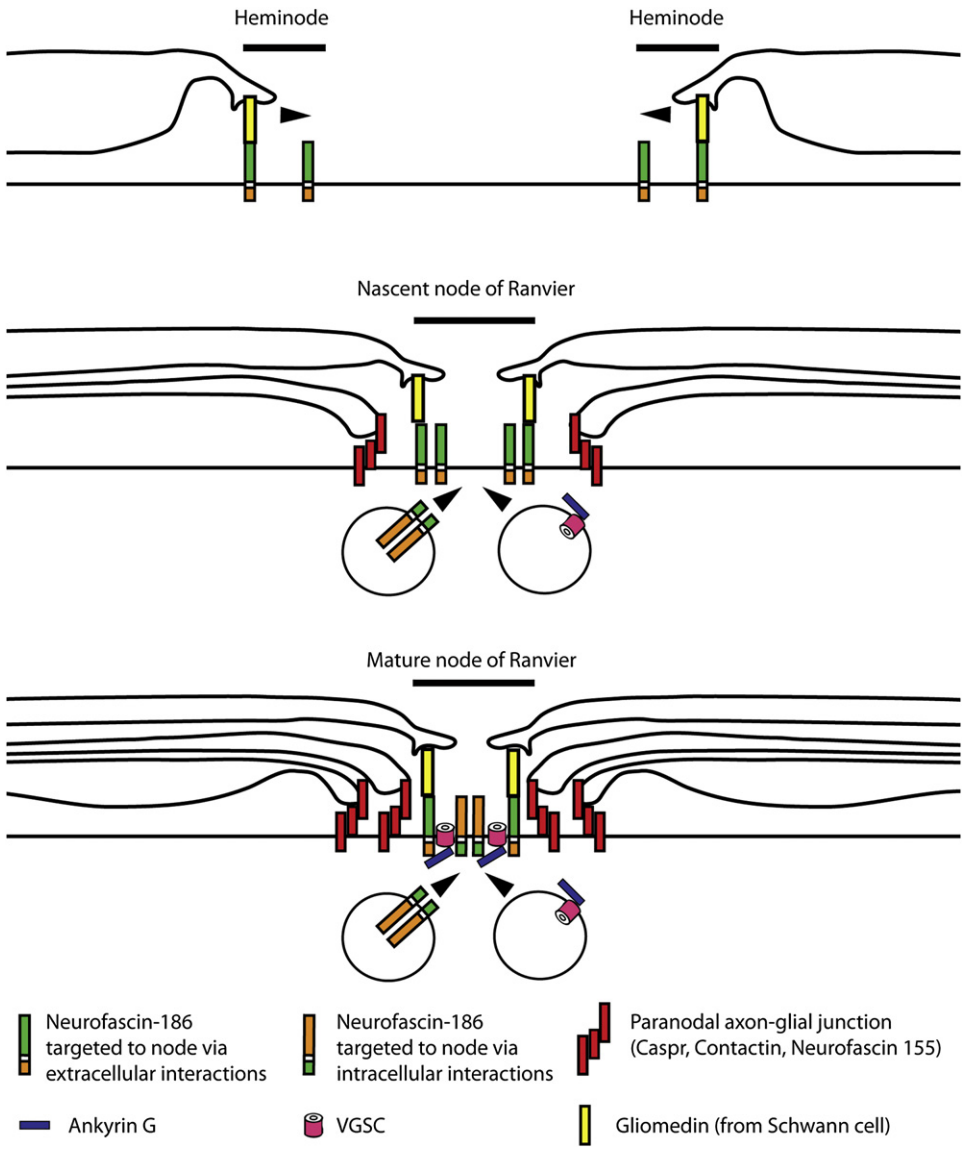

Figure 1. Targeting Proteins to Nodes of Ranvier

Top: Neurofascin-186 (see key) already in the axonal membrane is clustered at heminodes via its ectodomain, presumably by interactions with gliomedin on Schwann cells. The growth of Schwann cell processes toward one another actively clusters Neurofascin-186.

Middle: After the axon-glial junction forms, Neurofascin-186 is targeted to the node via vesicular transport, regulated by its C-terminal domain. Voltage-gated sodium channels (VGSCs) and the cytoskeletal component ankyrin G are also targeted to the node via vesicular transport.

Bottom: As the node matures there is a slow turnover of Neurofascin-186, which continues to be delivered to the node in a vesicle-dependent fashion, as are VGSCs and ankyrin G. Ankyrin G stabilizes Neurofascin-186 at the node.

ectodomain and gliomedin is likely to relocalize NF186 that is distributed along the membrane toward nodes, a process impaired in the absence of gliomedin. The later mechanism, which targets vesicles to the node after formation of the paranodal axon-glial junction, acts independently of gliomedin and heminodal clustering, and is sufficient to localize NF186 and VGSCs to nodes. The action of this second mechanism explains how functional nodes can form in gliomedin mutant mice. Interestingly, analyses of double mutant mice lacking both gliomedin and the paranodal CAM Caspr almost completely lack VGSCs at nodes of Ranvier (Feinberg et al., 2010). This may reflect disruption of both modes of NF186 trafficking defined by Zhang et al., which raises the possibility that the paranodal junction regulates the switch between NF186 trafficking modes.

It remains unclear whether there are also distinct modes of targeting key components to central nervous system (CNS) nodes of Ranvier. As in the PNS, NF186 is stably localized at CNS nodes and is necessary and sufficient for their long-term maintenance (Zonta et al., 2008, 2011; Thaxton et al., 2011). There are, however, apparent differences in the formation of nascent PNS and CNS nodes. In contrast to the PNS, there is evidence that early formation of an axon-glial junction, as oligodendrocyte processes grow along CNS axons, is

sufficient to cluster nodal molecules in the absence of NF186 (Zonta et al., 2008; but see Thaxton et al., 2011). This observation suggests that an early mechanism driven primarily by axon-glial interactions can also cluster molecules at nascent nodes in the CNS. This clustering can, however, occur in the absence of NF186 (Zonta et al., 2008) and gliomedin, which is not found at CNS nodes (Eshed et al., 2005), suggesting that there are important differences between the early modes of nascent node assembly in the PNS and CNS. The elegant experiments presented by Zhang et al. in *Neuron* have advanced our understanding of node formation in the PNS, and similar approaches that combine in vitro and in vivo manipulations with dynamic imaging of the various components of premyelinated and myelinated axons will also illuminate CNS node assembly and maintenance.

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All's Well that Ends Well: Arresting Cell Proliferation in Leaves

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The transition from cell proliferation to cell expansion is critical for determining leaf size. Andriankaja et al. (2012) demonstrate that in leaves of dicotyledonous plants, a basal proliferation zone is maintained for several days before abruptly disappearing, and that chloroplast differentiation is required to trigger the onset of cell expansion.

The final size and shape of plant leaves is under genetic control (Johnson and Lenhard, 2011). The genetic basis is evident from the uniformity of leaf size and shape within a given genotype, and, by contrast, the often large variation in leaf size among different genotypes, even when plants are grown in the same environment. Two cellular processes underlie leaf growth (Johnson and Lenhard, 2011): initially leaf cells proliferate, accumulating cytoplasmic mass, doubling in size and then dividing mitotically. Later on, after exiting the mitotic cycle, leaf cells grow by expansion, concomitant with a massive increase in the size of the central vacuole and often involving endoreduplication. The timing of the transition from proliferation to expansion is critical for setting final leaf size, as it determines how many cells form the "capital" for future expansion-driven growth (Poethig and Sussex, 1985). Indeed, many mutants affecting final leaf size appear to influence the timing of proliferation arrest (Mizukami and Fischer, 2000). Importantly, this arrest does not occur simultaneously throughout the leaf, but rather starts at the tip, and gradually moves to more basal cells (Donnelly et al., 1999). This process has led to the notion of a "proliferation-arrest front" that moves from the tip toward the base of the leaf. In fact, based on mutant and histological analyses, two successive arrest fronts have been proposed, with the first one terminating proliferation in most subepidermal cells excluding the vasculature and in epidermal pavement cells (i.e., ones not differentiating into trichomes and stomata), and the second one targeting specific cells like vascular or stomatal precursors that continue to proliferate for a longer time period (Nath et al., 2003; White, 2006).

In this issue of *Developmental Cell*, Andriankaja et al. subject this notion to closer scrutiny in *Arabidopsis thaliana* by quantifying the distribution of proliferating and expanding cells in the epidermis of the growing third leaf at daily intervals (Andriankaja et al., 2012). To do so, they develop an automated image-analysis algorithm that extracts cell shape parameters and uses these, based on an appropriate training data set, to classify cells as proliferating or expanding. After an initial phase when all cells are proliferating, Andriankaja et al. observe that expansion sets in at the very tip of the leaf. Over the next few days, the zone of proliferation at the base of the leaf blade actually increases in absolute terms both